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Techniques for Evaluating Bacillus thuringiensis and Spray Equipment for Aerial Application against Forest Defoliating Insects

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ABSTRACT

The most promising Dipel and Thuricide forestry spray formulations and spray equipment were studied to improve aerial application techniques and the effectiveness of *Bacillus thuringiensis* against forest insects. A fixed-wing aircraft, equipped with T8010 nozzles, was used to apply each formulation containing about 8 billion International Units of *Bacillus thuringiensis* per 2 gallons per acre to potted Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, trees. These trees were placed at 10-foot intervals across the spray swath and were subsequently bioassayed with test insects.

The spray formulations and spray equipment produced satisfactory drop-size spectra and spray deposit patterns. It was found that selected applications of *Bacillus thuringiensis* demonstrated high control potential for the Douglas-fir tussock moth, *Orgyia pseudotsugata* (McD.), and western spruce budworm, *Choristoneura occidentalis* Freeman. The mean corrected larval mortality of the Douglas-fir tussock moth was 88 percent for the 100-foot and 78 percent for the 200-foot swath widths using Dipel. For the Thuricide treatment, the mean corrected larval mortality of the Douglas-fir tussock moth was 94 percent for the 100-foot and 87 percent for the 200-foot swath widths; the mean corrected larval mortality of the western spruce budworm was 100 percent for the 100-foot and 98 percent for the 200-foot swath widths. Higher deposits of *Bacillus thuringiensis* almost completely prevented insect defoliation to the test trees.

Keywords: *Bacillus thuringiensis*, Douglas-fir tussock moth, *Orgyia pseudotsugata*, western spruce budworm, *Choristoneura occidentalis*, pesticide application methods, spraying (- pest control).

CONTENTS

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife -- if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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INTRODUCTION

Bacillus thuringiensis (*B.t.*) provides a potential for controlling important forest defoliating insects such as the gypsy moth, *Porthetria dispar* (L.), in the northeastern United States (Lewis et al. 1974) and spruce budworm, *Choristoneura fumiferana* (Clements), in eastern Canada (Tripp 1972, Smirnoff et al. 1973). The U.S. Forest Service has recently become interested in the possible use of *B.t.* as a replacement for DDT against two major western defoliators, the Douglas-fir tussock moth, *Orgyia pseudotsugata* McDunnough, and the western spruce budworm, *Choristoneura occidentalis* Freeman. However, improved spray formulations, dispersal equipment, and aerial application methods are needed to increase the effectiveness and reproducibility of field treatments (Lewis and Connola 1966, Boving et al. 1971, Falcon 1971).

Based on the results of laboratory evaluation of the physical properties of 12 *B.t.* candidate forestry formulations[1/] and on bioassay results with the larvae of the gypsy moth,[2/] the two most promising formulations were selected for this investigation. This paper describes techniques and presents the results of the study conducted in 1972 to evaluate the performance of Dipel and Thuricide spray formulations and spray equipment[3/] for increasing the effectiveness of aerial applications to control forest defoliating insects using the Douglas-fir tussock moth and the western spruce budworm as test insects.

[1/] Bohdan Maksymiuk and John Neisess, unpublished data.

[2/] Personal communication with N. R. Dubois, U.S. Forest Service, Northeastern Forest Experiment Station, Hamden, Conn. 06514.

[3/] Mention of proprietary products in this paper does not constitute endorsement by the U.S. Department of Agriculture.

MATERIALS AND METHODS

CALIBRATION OF SPRAY EQUIPMENT

A Piper Pawnee airplane was equipped with three stainless steel spray tanks (10 gallons each) and a stainless steel boom. The system was pressurized by compressed air. TeeJet diaphragm nozzles with 8010 nozzle tips were used. The nozzles were positioned symmetrically on the left and right booms (fig. 1) and were pointed forward and down at 45 degrees to the thrust line of the aircraft. For determining swath deposit patterns and physical and biological swath widths, the spray equipment was calibrated to deliver 2 gallons per acre (gpa) at 80 miles per hour airspeed and a 100-foot swath width. Reduced flow rates were used for an accurate determination of spray atomization at 40 and 60 pounds per square inch. The reduced rates (about 0.5 gpa) were used for preventing overlapping of spray drops.

Flow rates were determined for water and for Dipel formulation. The intent was to use water for future calibration in the field. The flow rate was determined for the outermost and innermost nozzles. Water or formulation was collected for 1-minute intervals from the two nozzles. The amount of water or the Dipel formulation collected was measured with a graduated cylinder. Three replicate calibrations were made with the water, and two with Dipel. A hand sprayer with an 8010 spray nozzle was used to determine calibrations for the Thuricide formulation.

FORMULATIONS

The most promising Dipel, Lot No. 3980-167A, and Thuricide, Lot No. 11164, experimental forestry formulations were selected for these tests (see footnote 1).

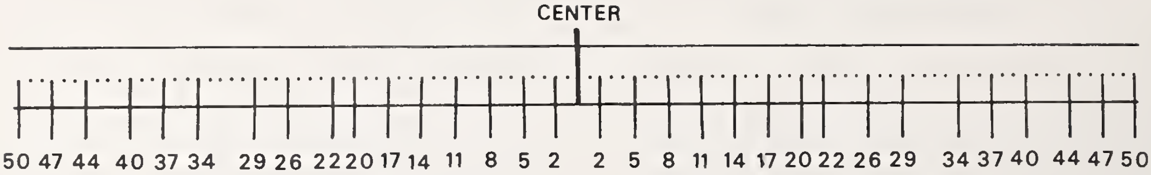

Figure 1.--Arrangement of T8010 nozzles along spray boom for testing Dipel and Thuricide formulations. This arrangement of 32 nozzles was used for determining swath deposit patterns for application rate of 2 gal/acre at 40 psi. 26 nozzles, numbered 2, 5, 8, 11, 14, 17, 20, 22, 26, 29, 34, 37, and 40, were used for determining swath deposit patterns for application rate of 2 gal/acre at 60 psi. 8 nozzles, numbered 8, 17, 26, and 34, were used for determining spray atomization at 40 and 60 psi.

The Dipel formulation contained: Dipel (Abbott Laboratories, North Chicago, Ill.), a wettable powder--227 grams; Cargill Insecticide Base (Cargill, Inc., Minneapolis, Minn.)--1 quart; propionic acid (Monsanto Co., St. Louis, Mo.)--7.57 milliliters; Santoquin (Monsanto Co., St. Louis, Mo.)--0.76 milliliter of 0.02 percent; Maywood formula (Maywood Chemical Co., Maywood, N.J.)--120 milliliters; and water--to make 1 gallon. The Thuricide formulation contained: Thuricide HPC (IMC Corp., Libertyville, Ill.), an emulsifiable concentrate--1 quart; Cargill Insecticide Base--1 quart; Chevron Spray Sticker (Chevron Chemical Co., San Francisco, Calif.)--4 fluid ounces; and water--to make 1 gallon.

The spray formulations contained a fluorescent tracer for deposit assessment (0.1 percent weight per volume Brilliant Sulphoflavine FFA, General Aniline and Film Corp., New York, N.Y.). Dipel contained 7.26 and Thuricide 8 billion International Units (IU) of potency per 2 gallons of finished spray to be applied per acre.

TEST ORGANISMS

Third- and fourth-instar larvae of the Douglas-fir tussock moth were used as test insects to determine the biological swath width for the Dipel formulations. Third- and fourth-instar larvae of the Douglas-fir tussock moth and the western spruce budworm were used for bioassay to determine the biological swath width for the Thuricide formulation.

Potted, greenhouse-grown Douglas-fir trees, *Pseudotsuga menziesii* (Mirb.) Franco (about 18 inches high), with the new flush of foliage, were used in all tests, including controls. Bioassay was used to determine biological swath width and patterns of larval mortality across the spray swath.

TEST CONDITIONS

Tests with the Dipel and Thuricide formulations were conducted at the 50-acre, open-ground airport test site where a rotating platform for sampling aerial sprays was installed (fig. 2). Meteorological conditions and application variables are shown in table 1. During the spray tests, wind speeds did not exceed 4 miles per hour. The following standardized procedure was used:

1. One potted tree, one white Kromekote card (4x5 inches), and two aluminum plates (6x6 inches) per each of 51 sampling stations were placed in line

Figure 2.--Rotating platform for sampling aerial sprays.

Table 1.--*Test parameters and meteorological conditions*

Bacillus thuringiensis and flight number[1/]	Number of nozzles, T8010	Gallons per acre	Spray pressure (lb/in^2)	Time (a.m.)	Temperature, °F		Wind		Platform orientation
					2 ft	4 ft	Mi/h	Direction	
Dipel:									
1	8	0.5	40	5:56	44	44	1.0	S	E to W
2	8	.5	60	6:25	44	44	1.5	SE	E to W
3	26	2.0	60	7:08	46	46	2.0	SE	E to W
4	32	2.0	40	7:55	48	47	4.0	S	N to S
Thuricide:									
1	8	.5	40	6:15	40	41	2.0	W	N to S
2	8	.5	58	6:40	46	45	1.0	SW	N to S
3	26	2.0	62	7:34	52	51	.5	W	N to S
4	32	2.0	40	8:30	58	56	1.0	SW	N to S

1/ 50-foot spraying height.

at 10-foot intervals on the rotating platform.

2. The platform was rotated to obtain either upwind or crosswind test flights as needed.

3. Each *B.t.* formulation was applied by an airplane flying at right angles to the platform; the indicated air speed was 80 miles per hour; and the spraying height was about 50 feet above the sampling platform. The spray was turned on 500 feet before the platform was reached and turned off 500 feet after it was passed.

4. A waiting period of 15-20 minutes allowed the spray to reach the surfaces of the samples (trees, cards, plates). A perforated plastic bag was then slipped over each potted tree before transport to the laboratory for bioassay.

5. Likewise, the Kromekote cards and aluminum plates were removed from each sampling station on the platform, placed in slotted boxes, and transported to the laboratory for spray deposit assessment.

BIOASSAY

In the laboratory, incisions were made in the plastic bags; 10 larvae were placed on the foliage of each of the field-labeled, potted trees; and the incisions were sealed with masking tape. In a similar manner, controls (10 larvae per each of 10 potted trees) had been set up before treatments to avoid possible contamination of foliage with *B.t.* All trees were kept at a temperature of about 22° C and watered to sustain the quality of foliage for larval feeding.

All treated and control plants were examined daily; and where the foliage had been depleted almost entirely, the larvae were transferred to untreated, potted trees. Six days after the treatment all living larvae were transferred to new untreated, potted trees, the dead larvae were removed from the plastic bags, and the mortality was recorded. Thirteen days after the treatment,the mortality was again determined and the remaining living larvae were transferred to artificial media in petri dishes for additional observation.

Frass produced by the larvae of the Douglas-fir tussock moth and spruce budworm was collected during the examination of larval mortalities in the

Thuricide treatment. Frass was oven-dried at 70° C for 24 hours and weighed.

SWATH WIDTH

Biological and physical swath widths for Dipel and Thuricide formulations were determined by plotting the percent of larval mortality together with the quantity of spray deposit across the spray swaths. Biologically effective swath widths and the pattern of larval mortality were determined only for finer spray atomization produced by a spray pressure of 60 pounds per square inch. Physical swath widths were also determined for the coarser spray atomization produced by a spray pressure of 40 pounds per square inch.

Spray deposit was expressed in terms of gallons per acre and the mean number of spray drops per square centimeter across the spray swaths. Physical swath widths and spray deposit distribution patterns were determined by fluorometric analysis of deposit (Yates and Akesson 1963) taken from two aluminum plates per each sampling station. The spray deposit was removed from each plate by washing with 10 milliliters of distilled water. The concentration of fluorescent tracer was determined by a Turner Model 430 Spectrofluorometer. The quantity of spray deposit, in gallons per acre, was determined by relating the amount of the fluorescent tracer to gallons per acre based on previously determined standard curves. The number of spray drops was determined from deposit appearing on a 4-square-centimeter central area of each Kromekote card.

SPRAY ATOMIZATION

Spray atomization, expressed as the volume median diameter (vmd),[4/] was determined from the deposit on the white Kromekote cards by using previously developed methods (Maksymiuk and Moore 1962, Maksymiuk 1964).

RESULTS AND DISCUSSION

DIPEL SPRAY FORMULATION

Calibration of spray equipment.--The calibration of the aircraft spray system is summarized in table 2. The flow rates of the finished Dipel formulation, tested at spray pressures of 40 and 60 pounds per square inch, were found to be essentially the same as for water. Therefore, water can be used for future calibrations of similar formulations. The

[4/] Volume median diameter (vmd) is the diameter of a drop which satisfies the requirement that half the volume of liquid be in drops smaller, and half in drops larger, than itself.

Table 2.--*Calibration of spray equipment (T8010 nozzles)*

Pressure (lb/in^2)	Flow rate of H_2O		Flow rate of Dipel	
	Center nozzle	Outer nozzle	Center nozzle	Outer nozzle
	Gallons per minute			
40	1.0010	0.9773	1.0270	0.9993
60	1.2349	1.2085	1.2305	1.2007

flow rates of the 8010 spray nozzles showed no practical variation regardless of their position on the spray boom. The uniform spray pressure should result in delivery of an accurate amount of spray. The flow rates of water and Dipel increased consistently with the increased spray pressure.

Evaluation of formulation.-- Two problems were encountered with the Dipel formulation. First, presence of cane fibers in the molasses formulation as it was received from the manufacturer might have plugged the nozzles of the spray equipment. This problem was remedied by filtering the formulation through a 50-mesh filter and by removing all the screens and filters in the spray equipment. It was very important to remove the screens and filters of the spray equipment; otherwise reduced flow rates could result. Second, the Dipel formulation tended to foam when vigorously agitated or pumped at high pressure into a spray tank. Foaming could be greatly reduced by pumping at a lower pressure, by using a hose that would not constrict the flow, or by stirring the foamy formulation.

Spray atomization.--Spray atomization data (vmd) are summarized in table 3. Increasing the spray pressure from 40 to 60 pounds per square inch resulted in the decreased vmd from 321 to 266 microns. The finer spray atomization produced more spray drops which can result in a higher density of deposit coverage, assuming no excessive evaporation and/or drift.

Biological and physical swaths.--Larval mortality for 100-foot and 200-foot swath widths are presented in table 3. The relationships between the larval mortalities (13 days) of the Douglas-fir tussock moth and the spray deposit in gallons per acre are shown graphically (fig. 3). These bioassay data show that the selected

Table 3.--*Summary of insect mortality and spray atomization data for Dipel and Thuricide formulations*

Formulation and test insect	Larval mortality for swath width[1/]		Vmd	
	100 feet (60 lb/in^2)	200 feet (60 lb/in^2)	40 lb/in^2	60 lb/in^2
	- - - - *Percent* - - - -		- - - - *Microns* - - - -	
Dipel:				
Douglas-fir tussock moth	88	78	321 ± 11[2/]	266 ± 25
Thuricide:				
Douglas-fir tussock moth	94	87	231 ± 37	199 ± 8
Western spruce budworm	100	98	231 ± 37	199 ± 8

[1/] Mortality data are corrected for controls using Abbott's formula (13 days).

[2/] Standard error of the mean of 3 determinations.

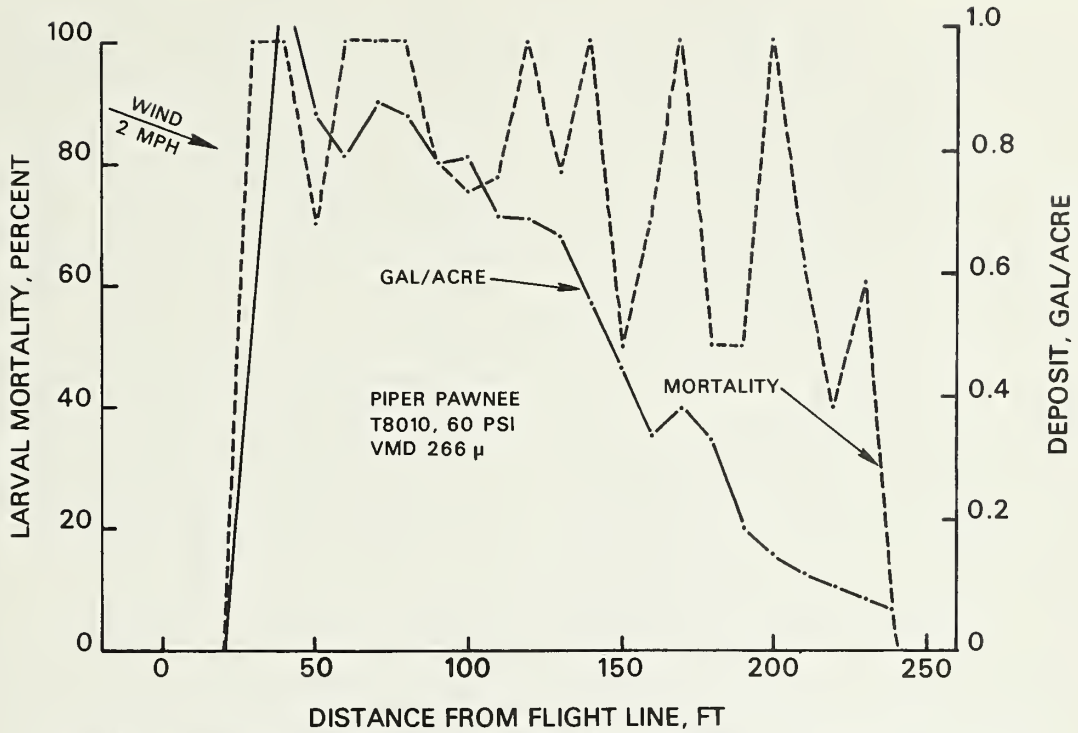

Figure 3.--Relationship between Douglas-fir tussock moth mortality and spray deposit in gallons per acre for Dipel.

spray equipment and Dipel formulation produce wide, biologically effective swaths.

The relationship between spray deposit in gpa and number of drops per square centimeter for the vmd of 266 microns (spray pressure of 60 pounds per square inch) is presented in figure 4. Similarly, the relationship between spray deposit in gpa and number of drops per square centimeter for the vmd of 321 microns (spray pressure of 40 pounds per square inch) is shown in figure 5. By comparing the swath widths (figs. 3 and 4 with fig. 5), it is evident that the finer atomization resulted in a wider swath width. This was despite the fact that there was less crosswind (2 miles per hour) for using spray atomization containing a vmd of 266 microns as compared to more crosswind (4 miles per hour) for using spray atomization having a vmd of 321 microns.

THURICIDE SPRAY FORMULATION

Calibration of spray equipment.--It was found that the flow rates of spray formulation from the hand sprayer closely approximated the aircraft calibrations when the same nozzles and operating spray pressures were employed. Therefore, calibrations for Thuricide spray formulations were made with a hand sprayer equipped with an 8010 nozzle at spray pressures of 40 and 60 pounds per square inch. The flow rates at 40 and 60 pounds per square inch were 0.9374 and 1.340 gallons per minute, respectively.

Evaluation of formulation.--No problems were encountered in the flowability of the Thuricide spray formulation

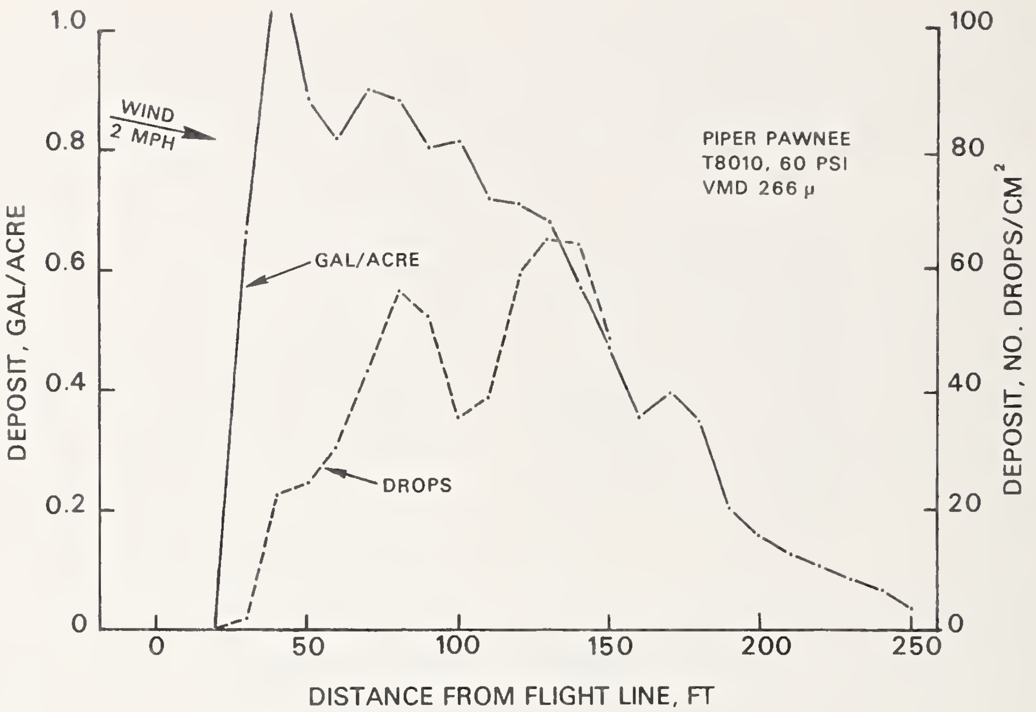

Figure 4.--Relationship between spray deposit expressed in gallons per acre and number of spray drops per square centimeter for Dipel; vmd of 266 microns.

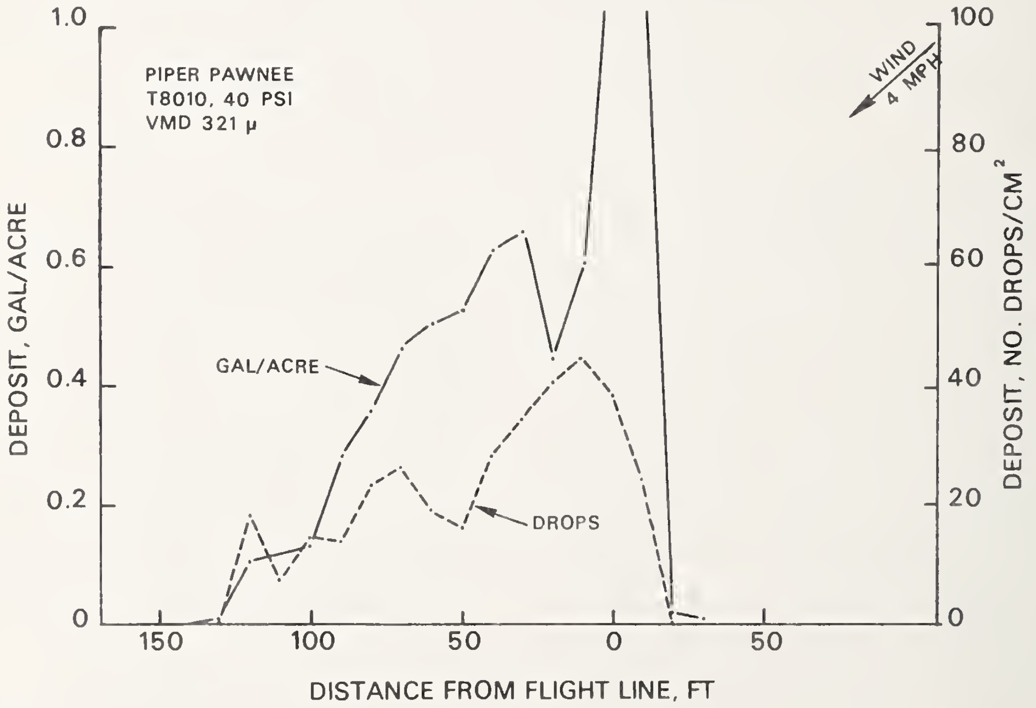

Figure 5.--Relationship between spray deposit expressed in gallons per acre and number of spray drops per square centimeter for Dipel; vmd of 321 microns.

through the spray equipment. Before being loaded into the aircraft, the formulation was filtered through a 50-mesh wire filter. No excessive amount of sediment was then found. Similarly, as with the Dipel formulation, all screens and filters were removed from the spray equipment to prevent possible plugging and irregular flow rates.

Spray atomization.--The spray atomization data for Thuricide are given in table 3 together with the Dipel formulation for convenient comparisons. Increasing the spray pressure from 40 to 60 pounds per square inch resulted in decreased vmd from 231 to 199 microns.

Biological and physical swaths.--Larval mortalities for 100-foot and 200-foot swath widths are given in table 3. The relationship between larval mortality (13 days) of the Douglas-fir tussock moth and spray deposit (gallons per acre) patterns across the spray swath is shown in figure 6. Comparable data for the distribution of mortality across the spray swath of the western spruce budworm are given in figure 7. These bioassay data show that the selected spray equipment and Thuricide *B.t.* spray formulation produced wide biologically effective swaths for the two test insects. It was found that the Thuricide formulation was more effective against the larvae of the western spruce budworm than against the larvae of the Douglas-fir tussock moth (figs. 6 and 7 and table 3).

The relationship between spray deposit in gallons per acre and number of spray drops per square centimeter for the vmd of 199 microns, across the spray swath, is illustrated in figure 8. The same relationship for vmd of 231 microns is depicted in figure 9. These graphs indicate satisfactory swath patterns in both the quantity and the density of spray deposits. However, finer atomization resulted in a higher drop density.

The relationships between larval mortality and larval frass of the Douglas-fir tussock moth and the western spruce budworm are depicted in figures 10 and 11, respectively. It was found that there was high negative correlation between insect mortality and frass production. There was virtually no larval feeding, as indicated by frass, on trees with high spray deposits (figs. 6 and 10 for the Douglas-fir tussock moth and figs. 7 and 11 for the western spruce budworm).

In conclusion, the reported techniques will be helpful in evaluating the performance of spray formulations and equipment for more effective aerial applications of microbial insecticides. The selected Dipel and Thuricide *B.t.* formulations and spray equipment performed satisfactorily. A 100-foot swath width is recommended for field use for a small, fixed-wing aircraft equipped with a conventional spray boom and nozzles. Results indicate that *B.t.* has a high potential for control of the Douglas-fir tussock moth and the western spruce budworm.

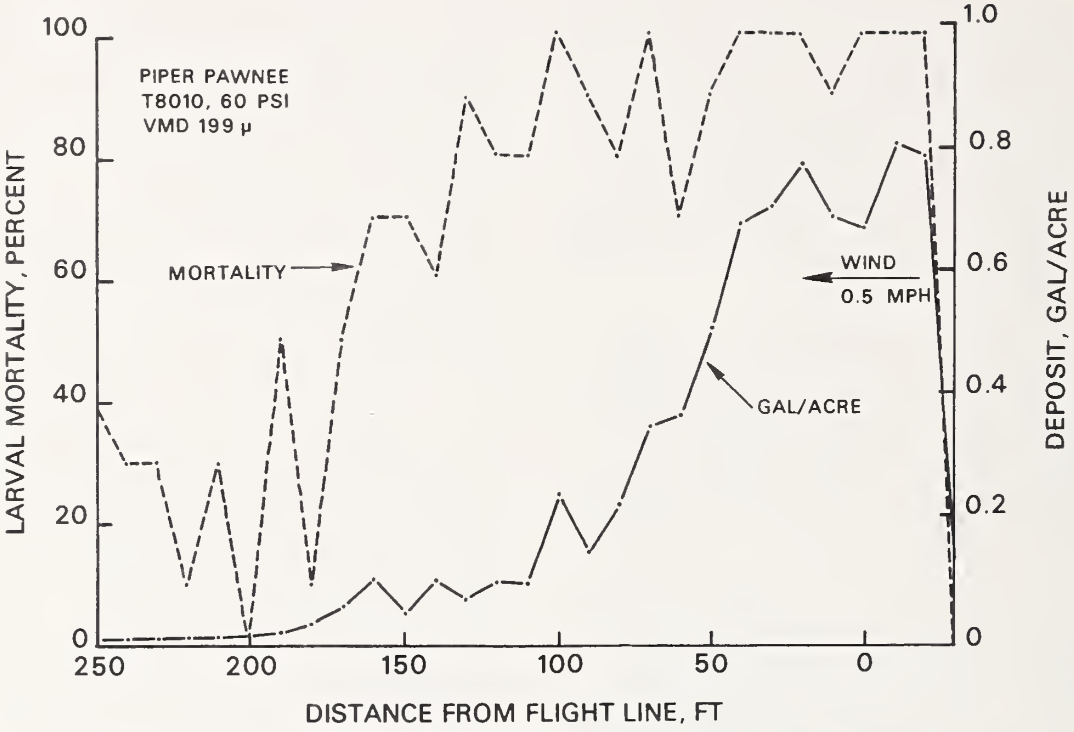

Figure 6.--Relationship between Douglas-fir tussock moth mortality and spray deposit in gallons per acre for Thuricide.

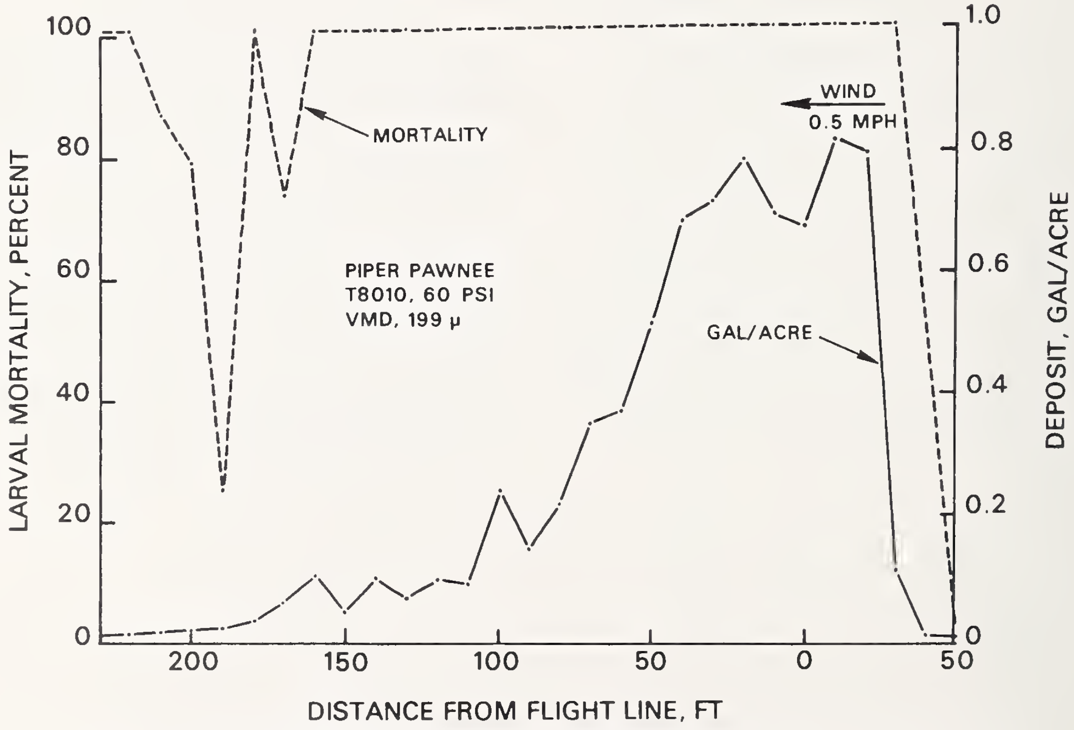

Figure 7.--Relationship between spruce budworm mortality and spray deposit in gallons per acre for Thuricide.

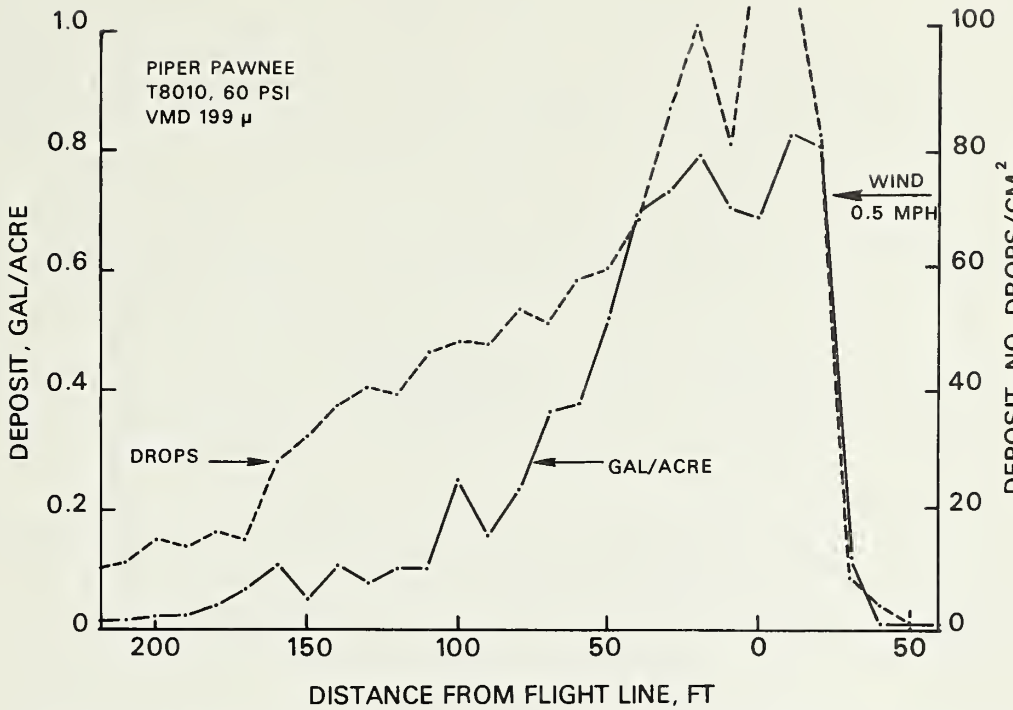

Figure 8.--Relationship between spray deposit expressed in gallons per acre and number of drops per square centimeter for Thuricide applied at 60 pounds per square inch.

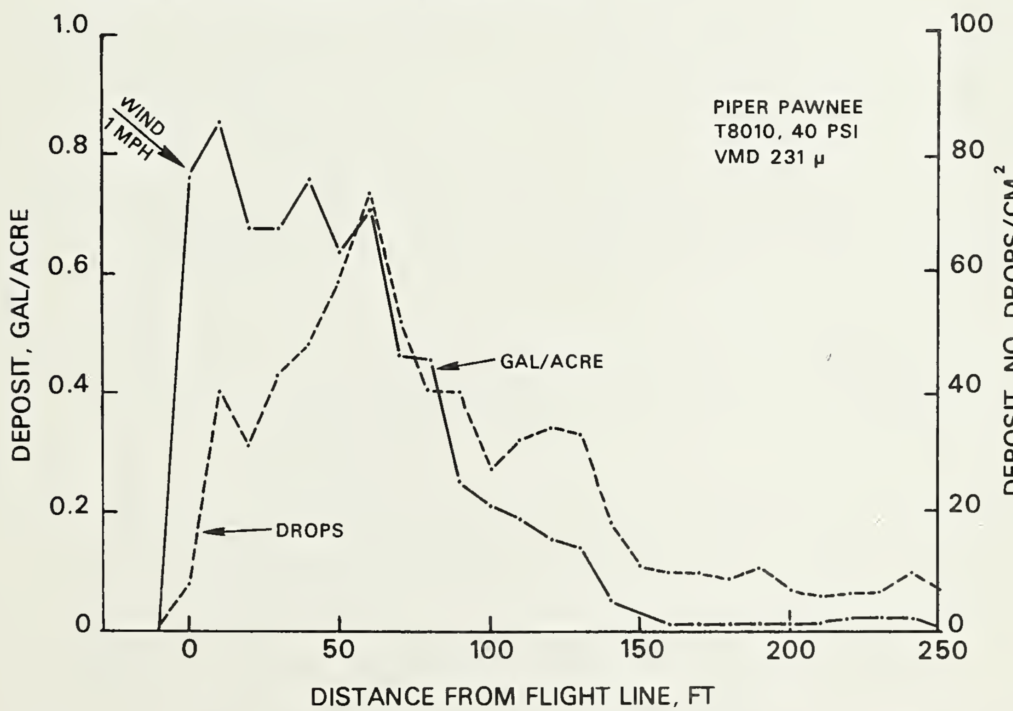

Figure 9.--Relationship between spray deposit expressed in gallons per acre and number of spray drops per square centimeter for Thuricide applied at 40 pounds per square inch.

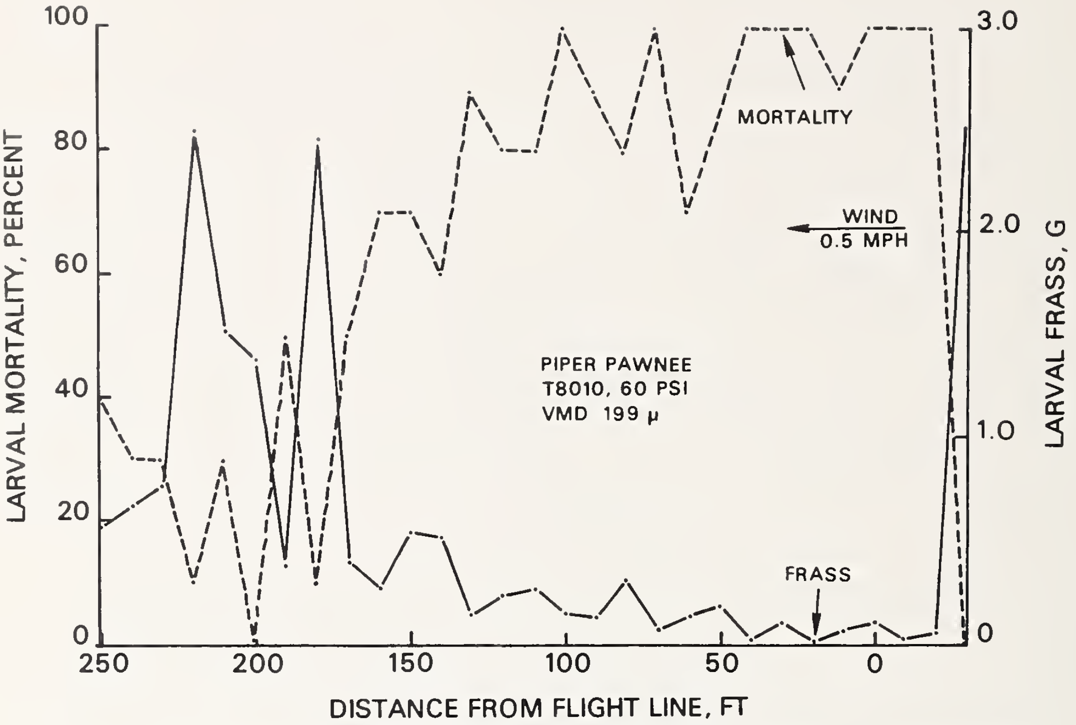

Figure 10.--Relationship between Douglas-fir tussock moth mortality and oven-dried frass (13 days) for Thuricide.

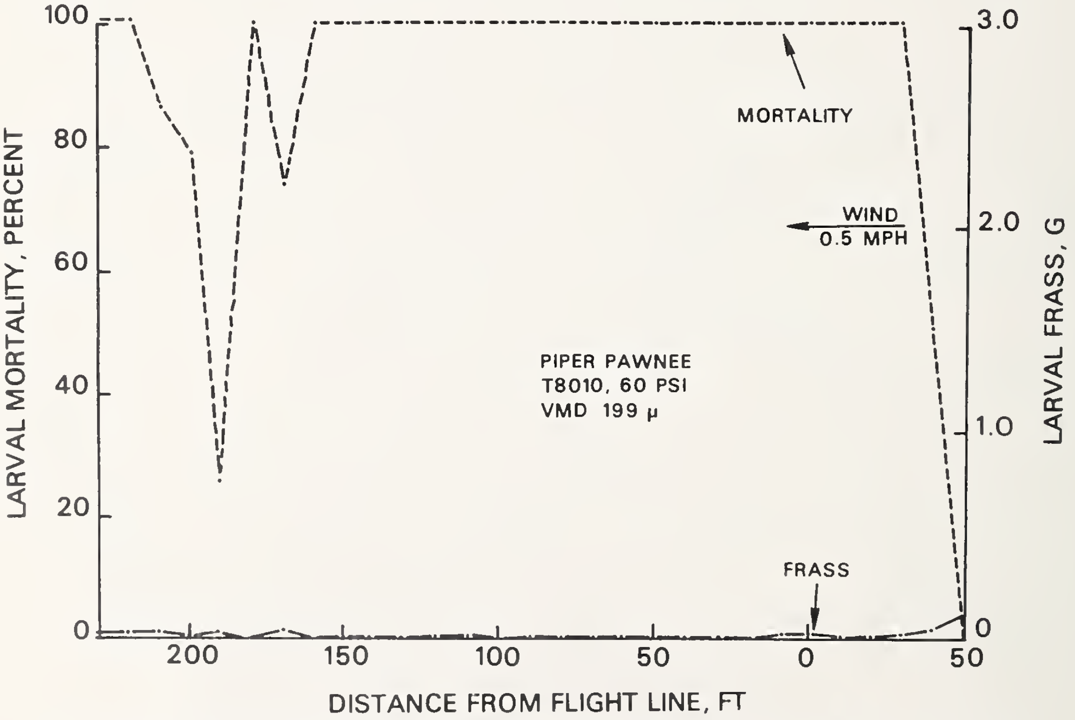

Figure 11.--Relationship between western spruce budworm mortality and oven-dried frass (13 days) for Thuricide.

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